HeLa cells co-transfected with MCU-V5 and Mfrn2-GFP, anti-GFP beads pulled down MCU-V5, and anti-V5 beads pulled down Mfrn2-GFP. COX-IV was not pulled down by either bead, indicating that the interaction between MCU and Mfrn2 was specific. In conclusion, Mfrn2 positively modulates Ru360-sensitive respiration-driven mitochondrial uptake of both Ca^{2+} and Fe^{2+}. Mfrn2 physically interacts with MCU and appears to be a component/regulator of the MCU complex. The mitochondrial calcium uniporter should more appropriately be called the mitochondrial Ca, Fe uniporter.

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S10.P17

Na^+-PPases: Strictly Na^+-specific transporters?

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Membrane pyrophosphatases (mPPases) are primary transporters that form Na^+ or H^+ concentration gradients (or both) using the energy they release upon PPi hydrolysis [1]. H^+-PPases have been discovered in prokaryotes, plants and protists; Na^+-PPases are found in bacteria and archaebacteria; and Na^+,H^+-PPases are mainly observed in bacteria of the human gastrointestinal tract [2]. mPPases function as funnel-like homodimers of ~75-kDa monomers [3]. mPPase overexpression in plants confers resistance to abiotic stresses, such as salinity and drought. Under physiological conditions, Na^+-PPases are strictly specific for Na^+ ions [1]. However, we discovered that the Na^+-PPases of Chlorobium limicola (Cl-PPase) and four other bacteria, as well as one species of archaebacteria, are able to transport H^+ ions at subphysiological Na^+ concentrations (<5 mM) in *Escherichia coli* inverted membrane vesicles [4]. The Na^+ ion transport rate, measured with $22Na^+$, remained constant over the Na^+ range 0.05 to 10 mM. H^+ transport was assayed with pH-responsive fluorescent dyes over the pH range 6.2 to 8.2 at increasing Na^+ concentrations. H^+ transport activity was absent without Na^+, was highest at 0.1 mM Na^+, and disappeared at concentrations of Na^+ greater than 5 mM. Mutational analysis revealed that Cl-PPase residues Glu-242, Ser-243 and Asn-677 are important in hydrolysis and H^+ transport, but do not control the Na^+ dependence of H^+ transport. Lys-681 substitution eliminated H^+-transport, but did not affect Na^+ transport. Each of these mutations decreased the enzyme's affinity for Na^+. These data further suggest that H^+-PPases have evolved from Na^+-PPases via subtle amino acid changes.

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S10.P18

Yeast phosphofructokinase-1 subunit Pfk2p is necessary for ph homeostasis and glucose-dependent V-ATPase reassembly: The role of glycolysis

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V-ATPases are conserved ATP-driven proton pumps that acidify organelles and energize secondary transport systems essential for lysosomal/vacuolar and endosomal functions. V-ATPase assembly and activity are glucose-dependent in yeast. Glucose depletion causes V-ATPase disassembly and its inactivation; glucose re-addition triggers reassembly and resumes proton transport and organelle acidification. Phosphofructokinase-1 is a key enzyme in the glycolytic pathway. The yeast ortholog of human phosphofructokinase-1 consists of two tetramers, each made of two subunits, Pfk1p and Pfk2p. We investigated the roles of the phosphofructokinase-1 subunits Pfk1p and Pfk2p for V-ATPase function. The pfk1Δ and pfk2Δ yeast mutants grew on glucose, indicating that they can metabolize glucose. In the wild-type cells, the subunits Pfk1p and Pfk2p co-immunoprecipitated with V-ATPase. Upon deletion of one subunit the other subunit retained binding to V-ATPase and the pfk1Δ and pfk2Δ cells assembled wild-type levels of V-ATPase pumps. The vacuolar lumen was alkalinized and the cytosol acidified in pfk1Δ and pfk2Δ cells, suggestive of impaired V-ATPase proton transport in vivo. These pH alterations were more dramatic in pfk2Δ, which also exhibited a partial vma growth phenotype. Binding of disassembled V-ATPase (V1 domain) to its assembly factor RAVE (subunit Rav1p) was 5-fold enhanced in pfk2Δ. RAVE-assisted glucose-dependent reassembly and/or glucose signals were disturbed; V-ATPase reassembly was significantly reduced after re-addition of glucose to glucose-deprived pfk2Δ cells. Normal V-ATPase function and regulation were rescued after increasing the concentration of glucose, which stimulated glycolysis in pfk2Δ. We concluded that V-ATPase proton transport, at steady state, and V-ATPase reassembly and reactivation, after glucose re-addition, are controlled by the glycolytic flow in yeast.

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Quantifying the importance of each site along the structure of mitochondrial carriers by monitoring single-nucleotide evolution

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Mitochondrial carriers (MCs) are proteins embedded in the inner mitochondrial membrane consisting of a tripartite structure, a three-fold pseudo-symmetry, related sequences, and similar folding whose main function is to catalyze the transport of various metabolites [1,2]. In this study, the evolutionary rate in vertebrates was screened at each of the approximately 50,000 nucleotides corresponding to the amino acids of the 53 human MCs. Using this information as a starting point, a scoring system was developed to quantify the evolutionary pressure acting on each site of the common MC structure and estimate its functional or structural relevance [3]. The degree of evolutionary selection varied greatly among all sites, but it was highly similar among the three symmetric positions in the tripartite structure, known as